HHS Public Access

Author manuscript

Anat Rec (Hoboken). Author manuscript; available in PMC 2020 January 01.

Published in final edited form as:

Anat Rec (Hoboken). 2019 January ; 302(1): 101–107. doi:10.1002/ar.23910.

Hand Factors in Cardiac Development

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Abstract

Congenital heart defects account for 1% of infant mortality and 10% of in-utero deaths. As the vertebrate embryo develops, multiple tissue types develop in tandem to morphologically pattern the functional heart. Underlying cardiac development is a network of transcription factors known to tightly control these morphological events. Members of the Twist family of basic helix-loop-helix (bHLH) transcription factors, Hand1 and Hand2, are essential to this process. The expression patterns and functional role of Hand factors in neural crest cells (NCC), endocardium, myocardium, and epicardium is indicative of their importance during cardiogenesis; however, to date, an extensive understanding of the transcriptional targets of Hand proteins and their overall mechanism of action remain unclear. In this review, we summarize the recent findings that further outline the crucial functions of Hand factors during heart development and in post-natal heart function.

Keywords

Heart; Cardiac development; Molecular Biology; Hand1; Hand2

CARDIAC MORPHOGENESIS

Congenital heart defects (CHDs) are the leading complication in pediatric mortalities (Ottaviani & Buja 2017). CHDs result from defects, genetic or environmental, of the developing heart in the first trimester of pregnancy. Cardiogenesis initiates as early as E6.5 in mice, when cardiac progenitor cells (CPCs) are specified in the anterior lateral plate mesoderm (Tam et al. 1997). As gastrulation proceeds, two molecularly distinct CPCs arise, the primary (PHF) and secondary heart field (SHF; Kelly et al. 2014). The cells of the PHF coalesce at the midline to form a linear tube that begins beating due to pacemaker activity in the venous pole (Sylva et al. 2014). The heart tube is composed of inner endothelial/endocardial cell layer and an outer myocardial layer. Proliferating SHF cells add to the outflow and inflow tracts causing the elongation of the heart tube and its subsequent looping in an asymmetric fashion by E9.5 (van den Berg et al. 2009; Vandenberg & Levin 2013; Francou et al. 2017).

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Multiple cell lineages contribute to the developing heart. NCCs are multipotent, migratory cells that arise from the dorsal neural tube (Noisa & Raivio 2014). Cardiac NCC migrate into the heart through the caudal pharyngeal arches (PAs) to septate the outflow tract, to form a patent aorta and pulmonary artery, as well as contribute to pulmonary and aortic valves (Keyte & Hutson 2012).

The epicardium forms the outer cell layer of the heart and is required for cardiac morphogenesis (Carmona et al. 2010). In mice, as cardiac looping begins, proepicardial villi from the pericardial side of the septum transversum initiate contact with the heart (Rodgers et al. 2008). By E10.5, these epicardial cell precursors cells cover the cardiac surface excluding the outflow tract (OFT). Around E12.5, a population of epicardial cells undergo secondary epithelial to mesenchymal transition (EMT) contributing to the coronary smooth muscle, cardiac fibroblasts and connective tissues of the pulmonary and aortic valves (Krainock et al. 2016).

The endocardium cell layer, in addition to providing a patent surface layer for circulation acts in conjunction with the myocardium as the heart develops. Myocardial-endocardial signaling has been shown to be critical for cardiac morphogenesis including, trabeculation, septation, and the development of the cardiac conduction system (Haack & Abdelilah-Seyfried 2016). In addition, populations of endocardial cells undergo EMT, populating the outflow tract (OFT) and atrial-ventricular cushions which ultimately contribute to the tricuspid and mitral valves (de Lange et al. 2004; MacGrogan et al. 2014). Endocardial contributions to coronary endothelium has also been proposed with some controversy (Tian et al. 2015).

In concert, these diverse cell populations all integrate to form a functional heart and each cell lineage relies on a finely tuned gene regulatory network driven by transcription factors modulating this complex integration. The basic Helix-Loop-Helix (bHLH) transcription factors Hand1 and Hand2 play key roles within the gene regulatory networks of NCC, epicardium, myocardium, and endocardium and in this review, our focus will be on recent studies involving Hand factors in these cell lineages.

HAND FACTORS

Hand factors are bHLH proteins that form homo- or hetero- dimers with bHLH partners and regulate gene expression (Firulli et al. 2000; Firulli et al. 2003; Firulli et al. 2005). They function as hetero- or homo- dimers and bind to consensus E - (CANNTG) or D – (CGNNTG) box sequences within the regulatory regions of gene targets (Massari & Murre 2000). Expression and lineage-tracing experiments have determined distinct and overlapping expression domains for these proteins in the developing cNCC, epicardium, myocardium, and endocardium. (Barnes & Firulli 2009).

Hand1 cardiac expression is first detectable in the mouse embryo at E8.5 in the posterior ventricle as well as a small domain of the developing OFT termed the myocardial cuff (Fig. 1; Barnes et al. 2011). As heart looping proceeds, *Hand1* expression is robust within the left ventricle (LV) between E9.5 –E13.5, is detected in both cNCC and SHF-derived myocardial

cuff of the OFT, and pericardium (Barbosa et al. 2007; Barnes et al. 2010). *Hand1* expression is not detectable within endocardial or epicardial cells; however, the epicardium, and all its derivatives, is *Hand1*-lineage derived (Barnes et al. 2010; Barnes et al. 2011).

Hand2 expression within cardiac and endocardial progenitors is detectable at E7.75 within the cardiac crescent (Fig. 1; Barnes et al. 2011). *Hand2* is robustly expressed within the SHF pharyngeal mesoderm that underlies and contributes to the growing heart tube (Tsuchihashi et al. 2011; Barnes et al. 2011). Through cardiac looping, low levels of *Hand2* myocardial expression is observed but endocardial expression is most robust (Fig. 1; VanDusen, Vincentz, et al. 2014). *Hand2* expression is also observed within cardiac NCC and myocardial cuff within the outflow tract as well as in the proepicardial organ and forming epicardium (VanDusen, Casanovas, et al. 2014). Systemic knockout of *Hand2* is embryonically lethal at E10.5 due to right ventricular (RV) hypoplasia and vascular malformations (Srivastava et al. 1997).

HAND FACTORS IN CARDIAC NEURAL CREST CELLS

cNCC migrate into the caudal PAs between E9.5 and E10.5 and play a key role in the patterning of the OFT (Yutzey & Kirby 2002). *Hand* gene expression is not detected in cNCC until post-migration (Vincentz et al. 2008) suggesting that their function lies in the patterning and differentiation of cNCC into OFT structures. Indeed, loss-of-function studies for *Hand1* and *Hand2* show an interesting functionally redundant role. When *Hand1* is deleted with *Wnt1-Cre*, resulting *Hand1* conditional knockouts are viable and fertile (Barbosa et al. 2007). However, reduction in *Hand2* gene dosage leads to mice born in expected ratios but die shortly after birth due to a failure to suckle (Barbosa et al. 2007). Examination of gene expression shows dysregulation of *Pax9, Msx2 and Prx2* in the developing mesenchyme. Loss of *Hand1* in conjunction with presence of only one copy of *Hand2* or deletion of *Hand2* in the pharyngeal arches leads to defects in neural crest derived distal midline mesenchyme development (Barbosa et al. 2007).

More recently, conditional knockout of *Hand2* using *Wnt1-Cre* display misalignment of the OFT, defective aortic arch arteries accompanied by ventricular septal defects (VSDs; Hendershot et al. 2008; Holler et al. 2010). Gene expression analysis of *Wnt1-Cre; Hand2* conditional knockouts show changes in genes involved in neural crest cell cycle and migration (Holler et al. 2010).

Loss of *Hand2* results in reduction of *Hand1* expression in cranial neural crest cells (Barron et al. 2011). However, *Hand1* expression within the cardiac neural crest cells is not dependent on Hand2 (Vincentz et al. 2016).

HAND FACTORS IN EPICARDIUM

Hand1 expression is observed in the septum transversum at E9.5 but *Hand1* expression is not observed in the epicardium (Barnes et al. 2011). Lineage tracing obtained from a *Cre* knocked into the *Hand1* allele showed that *Hand1*-lineage marks the proepicardium, epicardium, and epicardial derivatives – the cardiac fibroblasts and coronary smooth muscle (Barnes et al. 2010; Barnes et al. 2011). Thus, there is a temporal cascade of *Hand1* and

Hand2 expression; epicardial precursor cells express *Hand1* and as these cells migrate to the proepicardial organ, *Hand2* expression is turned on. When *Hand2* is conditionally deleted in the *Hand1* lineage (*H2CKO)*, epicardial expression of *Hand2* is lost indicating that within the epicardium, *Hand2* lies downstream of *Hand1* (Barnes et al. 2011). These *H2CKO* display defective epicardial EMT, decreased cardiac fibroblasts and nonfunctional coronary vasculature that leads to embryonic death by E16.5. Using a tamoxifen-inducible $WT1^{Cre}$ to ablate *Hand2* in epicardial cells leads to a similar phenotype (Barnes et al. 2011). During secondary epicardial EMT, *Pdgfra* is involved in cardiac fibroblast differentiation and *Pdgfrβ* is important for vascular smooth muscle differentiation (Krainock et al. 2016). In *H2CKOs*, *Pdgfra* is downregulated whereas *Pdgfrβ* expression is upregulated (Barnes et al. 2011). These data suggest an important role for Hand factors in the function of the epicardium.

HAND FACTORS IN CARDIOMYOCYTES

The Hand factors are excellent markers of ventricular identity; changes in their expression correlate with altered cardiac morphogenesis. For example, knockdown of *Tbx20* results in decreased expression of both *Hand1* and *Hand2* by E9.0 resulting in defective heart formation and hypoplasia of the RV and outflow tract (Takeuchi et al. 2005). Similarly, knockout of *Bmpr1a* in cardiac progenitors using *MesP1-Cre* leads to loss of *Hand1* expressing cells (Klaus et al. 2007). The requirement of patterning by Hand factors early in the developing embryo highlights their importance in the development of the ventricles.

Hand1 systemic knockout mice die by E9.5 due to defects in extra embryonic tissue and cardiac morphogenesis (Firulli et al. 1998; Riley et al. 1998). Heart development in *Hand1* homozygous null embryos is arrested due to failure of heart tube fusion at the caudal portion (Firulli et al. 1998). Conditional myocardial deletion of *Hand1* using the cardiomyocyte specific *αMHC-Cre* and *Nkx2.5-Cre* leads to reduced ventricular growth and maturation resulting in neonatal lethality (McFadden et al. 2005). Mice expressing a *Hand1* hypomorphic allele with 30% *Hand1* mRNA expression, survive longer than the systemic knockout, E10.5 to E12.5, exhibiting thin left ventricular myocardium along with extra-embryonic tissue defects (Firulli et al. 2010). In order to examine the role of *Hand1* in adult tissue post myocardial infarction, adult male mice haploinsufficient for *Hand1* were subjected to ligation of left anterior descending coronary artery (S. Lu et al. 2016). Post myocardial infarction, $Hand1^{+/-}$ mice show better heart function and decreased Matrix Metalloproteinase-9, MMP-9 expression, which might be a possible mechanism for protection post injury due to decreased cardiomyocyte apoptosis (S. Lu et al. 2016). In adult rats, post injury, *Hand1* expression is downregulated (Thattaliyath, Livi, et al. 2002).

Hand1 gain-of-function analysis using *MLC2V* promoter leads to enlarged RV and LV and loss of the intraventricular septum (Togi et al. 2004). Conditional *Hand1* gain-of-function in adult cardiomyocytes using a doxycycline inducible system results in arrhythmias (Breckenridge et al. 2009).

Recently the *cis*-regulatory elements that control *Hand1* expression in the myocardial cuff, cNCC, and, LV have been identified (Fig 2; Vincentz et al. 2017). Analysis of the conserved

regions upstream of the *Hand1* locus suggests putative enhancer DNA-binding sites (Fig. 2). A 744bp conserved enhancer region was isolated and shown to drive *Hand1* expression exclusively in the LV (Fig 2; Vincentz et al. 2017). This *Hand1* enhancer was used to drive *Cre* recombinase expression selectively in the LV, *Hand1*^{LV}*Cre*. Loss of the *Hand1* lineage LV cardiomyocytes using the *Hand1*^{LV}*Cre* with *Diphtheria Toxin A* (*R26R*^{DTA}) allele results in hypoplastic LV at E10.5. Surprisingly however, by E16.5, LV sizes are indistinguishable from controls, suggesting a compensatory growth by non-*Hand1* lineage cardiomyocytes in the developing LV. Since there is overlapping expression of *Hand2* in the myocardium by E11.5 and *Hand2* has been shown to compensate for loss of *Hand1*, further studies were performed ablating both *Hand1* and *Hand2* using *Hand1*^{LV}*Cre*. The loss of both Hand factors leads to a LV cardiomyocyte hyperplasia resulting in a severely occluded LV lumen, disorganized IVS, hyperplastic mitral valves and double outlet RV. The *Hand* LV knockout mice survive at a rate of 50% to birth with adults that have a severely compromised systolic function as measured by fractional shortening and ejection fraction. Cardiac marker analysis shows expansion of IVS (*Irx2* and *Dkk*) and compact zone (*Tbx20* and *Hey2*) marker genes. An increase in proliferation of cells in the LV trabeculae was shown to cause the LV hyperplasia phenotype. Ablation of *Hand1, Hand2* and both *Hand1/Hand2* with *Hand1*^{LV}*Cre* in the presence of *R26R*^{DTA} rescues this phenotype. This study demonstrates that the *Hand1* negative cardiomyocytes populating the LV are sufficient to generate a functional chamber; however, both *Hand1* and *Hand2* are required for normal LV development. Loss of both genes in the LV leads to a mispatterning of the LV and IVS as determined by marker gene expression.

Hand2 systemic knockout in mouse results in severe hypoplasia of the RV and dilated aortic sac by E9.5 and embryos die by E10.5 (Srivastava et al. 1997). These apoptotic RVs misexpress markers such as *Irx4* (Srivastava et al. 1997; Yamagishi et al. 2001). Overexpression of *Hand2* in ventricles leads to absence of IVS which is a further indication that *Hand2* expression is critical for the patterning of ventricles (Togi et al. 2006). Interestingly, Hand2 along with Gata4, Mef2c and Tbx5 are able to reprogram adult mouse cardiac fibroblasts into functional cardiomyocytes both *in vitro* and *in vivo* (Song et al. 2012).

When *Hand2* is ablated using *Nkx2.5-Cre*, cardiac looping takes places with the development of RV and LV, but mice die by E12.5 due to the lack of cardiomyocyte expansion (Tsuchihashi et al. 2011). This study also knocked out *Hand2* with *Isl1-Cre*, which is expressed in all early SHF cells, leading to embryonic death by E10.5 and severe hypoplasia of the right ventricle, similar to the systemic knockout. The investigators next used *Mef2c AHF-Cre*, which marks cells that give rise to the outflow track and right ventricle including the IVS, to ablate *Hand2*. These embryos die by E13.5, have thin RV myocardium and VSDs. Hand2 is required for the early survival of SHF progenitors and loss of *Hand2* in these populations results in defective myocardium and IVS (Tsuchihashi et al. 2011).

Hand2 has been shown to regulate *Nppa* independent of its DNA binding ability (Thattaliyath, Firulli, et al. 2002). Hand2 ChIP-seq analysis was performed using a mouse

line targetted with 3xFLAG epitope tag to the N-terminus of Hand2 by Laurent et al to study transcriptional targets of Hand2 on a genome wide scale (Laurent et al. 2017).

Gene ontology analysis of the Hand2 cistrome and whole mount *in-situ* hybridization showed that genes involved in EMT, specifically *Has2* and *Snai1* to be direct targets of Hand2 at E9.5 in the heart. The *Snai1* locus was shown to contain two Hand2 responsive *cis*-regulatory elements that drive *Snai1* expression in the heart (Laurent et al. 2017).

Spatial and temporal regulation of *Hand2* expression was shown to involve the microRNA, *miR-1* (Zhao et al. 2005). In these experiments, perturbation of *Hand2* expression by knocking out *miR-1* led to changes in proliferation of ventricular myocytes. *Hand2* mediated RV maturation depends on activation of enhancer regions in the *Hand2* promoter that bind GATA4 (McFadden et al. 2000). This is further confirmed by conditional deletion of *Gata4* in the heart with $Nkx2.5^{Cre}$ which leads to the loss of expression of *Hand2* (Zeisberg et al. 2005). More recently, *upperhand* been shown to directly regulate *Hand2* expression in *cis* during development (Anderson et al. 2016). Systemic knockout of *upperhand* leads to loss of *Hand2* expression in the ventricles and outflow tract.

In the adult, cardiac dysfunction is most commonly attributed to cardiomyopathies that impair LV function such as hypoplastic left heart syndrome (HLHS) and LV non-compaction (LVNC). The Hand factors have been implicated in the etiology of these diseases (Jiang et al. 2014). Formalin fixed heart tissue samples from human patients with HLHS were reported to contain protein mutations in *Hand1* (Reamon-Buettner et al. 2008; Wang et al. 2011). However, further analysis from fresh frozen tissue from HLHS patients did not recapitulate this finding (Esposito et al. 2011; Durbin et al. 2017). Analysis of the molecular consequences of this mutation in mice was conducted by generating a transgenic animal with mutant *Hand1* allele that has a nucleotide deletion at codon 126, causing a frameshift mutation leading to termination after 13 amino acids with a stop flox cassette that allows conditional activation (Firulli, Toolan, et al. 2017). When crossed with $Nkx2.5^{Cre}$, embryos die by E14.5 accompanied by cardiac outflow tract and IVS abnormalities. Using *αMHC-Cre* or *Mef2c AHF-Cre* to express mutant protein in cardiomyocytes results in reduced phenotype and limited viability. As was determined by examination of fresh frozen human tissue samples (Esposito et al. 2011; Durbin et al. 2017), LVs of *Hand1A126FS* mutant mice are not hypoplastic (Firulli, Toolan, et al. 2017).

Single nucleotide polymorphisms and haploinsufficiency at the *Hand1* locus are associated with CHDs (Starkovich et al. 2016). Human CHDs have been linked with defects in *Hand2* expression as well (Tamura et al. 2013; C.-X. Lu et al. 2016). *Hand2* has also been shown to be induced in post-natal cardiomyopathies and required to drive pressure overload induced cardiac remodeling (Thattaliyath, Livi, et al. 2002; Dirkx et al. 2013). More extensive examination is required to determine the exact molecular interactions leading to these CHD phenotypes. The critical role that Hand1 and Hand2 play in early development would seem to preclude mutations in the protein itself. It is more likely that changes in the enhancer regions controlling the expression of these genes are responsible for human CHDs.

HAND FACTORS IN ENDOCARDIUM

Conditional knockout of *Hand1* using *αMHC-Cre* displayed defects in endocardial cushions (McFadden et al. 2005). However, since lineage trace analysis do not identify *Hand1* expressing cells in the endocardium (Barnes et al. 2010), these phenotypes are most likely due to defects in myocardial signaling.

Hand2 is strongly expressed in the endocardium (Fig. 1; Barnes et al. 2011). In mice where the Hand2 DNA binding domain is perturbed, at E11.5 show disorganized endocardial cushions with reduced trabeculation and are lethal by E12.5 (Liu et al. 2009). *Hand2* ablation using the *Mef2c AHF-Cre*, which marks SHF progenitors that contribute to the endocardium, shows defects in the tricuspid valve, tricuspid atresia (Tsuchihashi et al. 2011). However, cardiomyocyte specific loss of *Hand2* does not show this phenotype (Tsuchihashi et al. 2011). Ablating *Hand2* specifically in the endocardium using either the *Tie2-Cre* or *Nfatc1*^{Cre} (*H2CKO)* leads to tricuspid atresia and double inlet LV (VanDusen, Casanovas, et al. 2014). This study also shows that loss of *Hand2* in endocardial populations resulted in reduced trabeculae in the developing ventricles as assessed by expression of *Bmp10*. Hand2 function within the endocardium lies within the Notch signaling pathway as *Hand2* is down-regulated in the endocardium of *RBPJκ* knockout mice, the target transcription factor for canonical Notch signaling. An endocardial specific conditional knockout generated using *Tie2-Cre* deletion of *EphrinB2* (*EfnB2*), a direct Notch target also leads to a loss of *Hand2* expression. However, conditionally ablating *Hand2* using the same *Cre* does not lead to a change in *EfnB2* expression. Although, the trabeculation growth factor, *Neuregulin1* (*Nrg1)*, also an *EfnB2* target, is downregulated in *H2CKO* as assessed by ISH and qRT-PCR and is directly regulated by *Hand2* as shown by *Nrg1* promoter analysis. Conditional gain-of-function of *Hand2* in *Nfatc1-Cre* conditional knockouts of *EfnB2* leads to a rescue of *Nrg1* and *Bmp10* positive trabeculae. Thus, these data demonstrated that *Hand2* acts downstream of *EfnB2* but upstream of *Nrg1* to regulate endocardial development. *H2CKO* also exhibits a hypervascularization phenotype (VanDusen, Casanovas, et al. 2014). Genes involved in vascular differentiation, specifically components of *Vegf* signaling, were misregulated with increased expression of *VegfR3*, *Vegfa*, and *Lyve-1*. Direct regulation by *Hand2* was shown by promoter analysis of a *Vegf* signaling cofactor, *Nrp1* using ChIP-seq and luciferase assays. These results show the importance of *Hand2* in the development of the endothelial component of coronary vasculature.

The role that Hand factors play in development is well studied and some of the molecular pathways that these transcription factors are involved in have recently been elucidated. Although not discussed in this review, Hand factors also play an important role in other tissue types, most notably the limb (Osterwalder et al. 2014; Firulli, Milliar, et al. 2017) and craniofacial tissues (Firulli et al. 2014). The gene regulatory networks that involve Hand factors also remain an area of focus. Further work looking at the mechanism of action of Hand factors is necessary to fully elucidate their biological function.

ACKNOWLEDGEMENTS

We would like to thank the members of the Firulli lab for their helpful discussion. Work in the Firulli lab is supported by NIH. Infrastructural support to the Herman B Wells Center for Pediatric Research is in part supported by The Riley Children's Foundation, Division of Pediatric Cardiology and the Carrolton Buehl McCulloch Chair of Pediatrics. We would like to thank all the researchers whose work has been cited here.

REFERENCES

Anderson KM, Anderson DM, McAnally JR, Shelton JM, Bassel-Duby R, Olson EN. 2016 Transcription of the non-coding RNA upperhand controls Hand2 expression and heart development. Nature. 539:433–436. [PubMed: 27783597]

Barbosa AC, Funato N, Chapman S, McKee MD, Richardson JA, Olson EN, Yanagisawa H. 2007 Hand transcription factors cooperatively regulate development of the distal midline mesenchyme. Dev Biol. 310:154–168. [PubMed: 17764670]

Barnes RM, Firulli AB. 2009 A twist of insight - the role of Twist-family bHLH factors in development. Int J Dev Biol. 53:909–924. [PubMed: 19378251]

Barnes RM, Firulli BA, Conway SJ, Vincentz JW, Firulli AB. 2010 Analysis of the Hand1 cell lineage reveals novel contributions to cardiovascular, neural crest, extra-embryonic, and lateral mesoderm derivatives. Dev Dyn. 239:3086–3097. [PubMed: 20882677]

Barnes RM, Firulli BA, VanDusen NJ, Morikawa Y, Conway SJ, Cserjesi P, Vincentz JW, Firulli AB. 2011 Hand2 loss-of-function in Hand1-expressing cells reveals distinct roles in epicardial and coronary vessel development. Circ Res. 108:940–949. [PubMed: 21350214]

Barron F, Woods C, Kuhn K, Bishop J, Howard MJ, Clouthier DE. 2011 Downregulation of Dlx5 and Dlx6 expression by Hand2 is essential for initiation of tongue morphogenesis. Development. 138:2249–2259. [PubMed: 21558373]

Breckenridge RA, Zuberi Z, Gomes J, Orford R, Dupays L, Felkin LE, Clark JE, Magee AI, Ehler E, Birks EJ, et al. 2009 Overexpression of the transcription factor Hand1 causes predisposition towards arrhythmia in mice. J Mol Cell Cardiol. 47:133–141. [PubMed: 19376125]

Carmona R, Guadix JA, Cano E, Ruiz-Villalba A, Portillo-Sánchez V, Pérez-Pomares JM, Muñoz-Chápuli R. 2010 The embryonic epicardium: an essential element of cardiac development. J Cell Mol Med. 14:2066–2072. [PubMed: 20477903]

de Lange FJ, Moorman AFM, Anderson RH, Männer J, Soufan AT, de Gier-de Vries C, Schneider MD, Webb S, van den Hoff MJB, Christoffels VM. 2004 Lineage and Morphogenetic Analysis of the Cardiac Valves. Circ Res. 95:645–654. [PubMed: 15297379]

Dirkx E, Gladka MM, Philippen LE, Armand A-S, Kinet V, Leptidis S, Azzouzi el H, Salic K, Bourajjaj M, da Silva GJJ, et al. 2013 Nfat and miR-25 cooperate to reactivate the transcription factor Hand2 in heart failure. Nat Cell Biol. 15:1282–1293. [PubMed: 24161931]

Durbin MD, Cadar AG, Williams CH, Guo Y, Bichell DP, Su YR, Hong CC. 2017 Hypoplastic Left Heart Syndrome Sequencing Reveals a Novel NOTCH1 Mutation in a Family with Single Ventricle Defects. Pediatr Cardiol. 38:1232–1240. [PubMed: 28608148]

Esposito G, Butler TL, Blue GM, Cole AD, Sholler GF, Kirk EP, Grossfeld P, Perryman BM, Harvey RP, Winlaw DS. 2011 Somatic mutations in NKX2–5, GATA4, and HAND1 are not a common cause of tetralogy of Fallot or hypoplastic left heart. Am J Med Genet. 155A:2416–2421. [PubMed: 22043484]

Firulli AB, McFadden DG, Lin Q, Srivastava D, Olson EN. 1998 Heart and extra-embryonic mesodermal defects in mouse embryos lacking the bHLH transcription factor Hand1. Nat Genet. 18:266–270. [PubMed: 9500550]

Firulli BA, Fuchs RK, Vincentz JW, Clouthier DE, Firulli AB. 2014 Hand1 phosphoregulation within the distal arch neural crest is essential for craniofacial morphogenesis. Development. 141:3050–3061. [PubMed: 25053435]

Firulli BA, Hadzic DB, McDaid JR, Firulli AB. 2000 The basic helix-loop-helix transcription factors dHAND and eHAND exhibit dimerization characteristics that suggest complex regulation of function. J Biol Chem. 275:33567–33573. [PubMed: 10924525]

Firulli BA, Howard MJ, McDaid JR, McIlreavey L, Dionne KM, Centonze VE, Cserjesi P, Virshup DM, Firulli AB. 2003 PKA, PKC, and the protein phosphatase 2A influence HAND factor function: a mechanism for tissue-specific transcriptional regulation. Mol Cell. 12:1225–1237. [PubMed: 14636580]

Firulli BA, Krawchuk D, Centonze VE, Vargesson N, Virshup DM, Conway SJ, Cserjesi P, Laufer E, Firulli AB. 2005 Altered Twist1 and Hand2 dimerization is associated with Saethre-Chotzen syndrome and limb abnormalities. Nat Genet. 37:373–381. [PubMed: 15735646]

Firulli BA, McConville DP, Byers JS, Vincentz JW, Barnes RM, Firulli AB. 2010 Analysis of a Hand1 hypomorphic allele reveals a critical threshold for embryonic viability. Dev Dyn. 239:2748–2760. [PubMed: 20737509]

Firulli BA, Milliar H, Toolan KP, Harkin J, Fuchs RK, Robling AG, Firulli AB. 2017 Defective Hand1 phosphoregulation uncovers essential roles for Hand1 in limb morphogenesis. Development. 144:2480–2489. [PubMed: 28576769]

Firulli BA, Toolan KP, Harkin J, Millar H, Pineda S, Firulli AB. 2017 The HAND1 frameshift A126FS mutation does not cause hypoplastic left heart syndrome in mice. Cardiovasc Res.

Francou A, De Bono C, Kelly RG. 2017 Epithelial tension in the second heart field promotes mouse heart tube elongation. Nat Commun. 8:14770. [PubMed: 28357999]

Haack T, Abdelilah-Seyfried S. 2016 The force within: endocardial development, mechanotransduction and signalling during cardiac morphogenesis. Development. 143:373–386. [PubMed: 26839341]

Hendershot TJ, Liu H, Clouthier DE, Shepherd IT, Coppola E, Studer M, Firulli AB, Pittman DL, Howard MJ. 2008 Conditional deletion of Hand2 reveals critical functions in neurogenesis and cell type-specific gene expression for development of neural crest-derived noradrenergic sympathetic ganglion neurons. Dev Biol. 319:179–191. [PubMed: 18501887]

Holler KL, Hendershot TJ, Troy SE, Vincentz JW, Firulli AB, Howard MJ. 2010 Targeted deletion of Hand2 in cardiac neural crest-derived cells influences cardiac gene expression and outflow tract development. Dev Biol. 341:291–304. [PubMed: 20144608]

Jiang Y, Habibollah S, Tilgner K, Collin J, Barta T, Al-Aama JY, Tesarov L, Hussain R, Trafford AW, Kirkwood G, et al. 2014 An induced pluripotent stem cell model of hypoplastic left heart syndrome (HLHS) reveals multiple expression and functional differences in HLHS-derived cardiac myocytes. Stem Cells Transl Med. 3:416–423. [PubMed: 24591732]

Kelly RG, Buckingham ME, Moorman AF. 2014 Heart fields and cardiac morphogenesis. Cold Spring Harb Perspect Med. 4:a015750. [PubMed: 25274757]

Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D. 2002 The human genome browser at UCSC. Genome Res. 12:996–1006. [PubMed: 12045153]

Keyte A, Hutson MR. 2012 The neural crest in cardiac congenital anomalies. Differentiation. 84:25–40. [PubMed: 22595346]

Klaus A, Saga Y, Taketo MM, Tzahor E, Birchmeier W. 2007 Distinct roles of Wnt/beta-catenin and Bmp signaling during early cardiogenesis. Proc Natl Acad Sci USA. 104:18531–18536. [PubMed: 18000065]

Krainock M, Toubat O, Danopoulos S, Beckham A, Warburton D, Kim R. 2016 Epicardial Epithelial-to-Mesenchymal Transition in Heart Development and Disease. J Clin Med. 5:27.

Laurent F, Girdziusaite A, Gamart J, Barozzi I, Osterwalder M, Akiyama JA, Lincoln J, Lopez-Rios J, Visel A, Zuniga A, Zeller R. 2017 HAND2 Target Gene Regulatory Networks Control Atrioventricular Canal and Cardiac Valve Development. Cell Rep. 19:1602–1613. [PubMed: 28538179]

Liu N, Barbosa AC, Chapman SL, Bezprozvannaya S, Qi X, Richardson JA, Yanagisawa H, Olson EN. 2009 DNA binding-dependent and -independent functions of the Hand2 transcription factor during mouse embryogenesis. Development. 136:933–942. [PubMed: 19211672]

Lu C-X, Gong H-R, Liu X-Y, Wang J, Zhao C-M, Huang R-T, Xue S, Yang Y-Q. 2016 A novel HAND2 loss-of-function mutation responsible for tetralogy of Fallot. Int J Mol Med. 37:445–451. [PubMed: 26676105]

Lu S, Du P, Shan C, Wang Y, Ma C, Dong J. 2016 Haploinsufficiency of Hand1 improves mice survival after acute myocardial infarction through preventing cardiac rupture. Biochem Biophys Res Commun. 478:1726–1731. [PubMed: 27601324]

MacGrogan D, Luxán G, Driessen-Mol A, Bouten C, Baaijens F, La Pompa De JL. 2014 How to Make a Heart Valve: From Embryonic Development to Bioengineering of Living Valve Substitutes. Cold Spring Harb Perspect Med. 4:a013912–a013912. [PubMed: 25368013]

Massari ME, Murre C. 2000 Helix-loop-helix proteins: regulators of transcription in eucaryotic organisms. Mol Cell Biol. 20:429–440. [PubMed: 10611221]

McFadden DG, Barbosa AC, Richardson JA, Schneider MD, Srivastava D, Olson EN. 2005 The Hand1 and Hand2 transcription factors regulate expansion of the embryonic cardiac ventricles in a gene dosage-dependent manner. Development. 132:189–201. [PubMed: 15576406]

McFadden DG, Charité J, Richardson JA, Srivastava D, Firulli AB, Olson EN. 2000 A GATA-dependent right ventricular enhancer controls dHAND transcription in the developing heart. Development. 127:5331–5341. [PubMed: 11076755]

Noisa P, Raivio T. 2014 Neural crest cells: From developmental biology to clinical interventions. Birth Defects Res C Embryo Today. 102:263–274. [PubMed: 25226872]

Osterwalder M, Speziale D, Shoukry M, Mohan R, Ivanek R, Kohler M, Beisel C, Wen X, Scales SJ, Christoffels VM, et al. 2014 HAND2 targets define a network of transcriptional regulators that compartmentalize the early limb bud mesenchyme. Dev Cell. 31:345–357. [PubMed: 25453830]

Ottaviani G, Buja LM. 2017 Update on congenital heart disease and sudden infant/perinatal death: from history to future trends. J Clin Pathol. 70:555–562. [PubMed: 28450386]

Reamon-Buettner SM, Ciribilli Y, Inga A, Borlak J. 2008 A loss-of-function mutation in the binding domain of HAND1 predicts hypoplasia of the human hearts. Hum Mol Genet. 17:1397–1405. [PubMed: 18276607]

Riley P, Anson-Cartwright L, Cross JC. 1998 The Hand1 bHLH transcription factor is essential for placentation and cardiac morphogenesis. Nat Genet. 18:271–275. [PubMed: 9500551]

Rodgers LS, Lalani S, Runyan RB, Camenisch TD. 2008 Differential growth and multicellular villi direct proepicardial translocation to the developing mouse heart. Dev Dyn. 237:145–152. [PubMed: 18058923]

Song K, Nam Y-J, Luo X, Qi X, Tan W, Huang GN, Acharya A, Smith CL, Tallquist MD, Neilson EG, et al. 2012 Heart repair by reprogramming non-myocytes with cardiac transcription factors. Nature. 485:599–604. [PubMed: 22660318]

Srivastava D, Thomas T, Lin Q, Kirby ML, Brown D, Olson EN. 1997 Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, dHAND. Nat Genet. 16:154–160. [PubMed: 9171826]

Starkovich M, Lalani SR, Mercer CL, Scott DA. 2016 Chromosome 5q33 deletions associated with congenital heart defects. Am J Med Genet. 170:3338–3342. [PubMed: 27589475]

Sylva M, van den Hoff MJB, Moorman AFM. 2014 Development of the human heart. Am J Med Genet. 164A:1347–1371. [PubMed: 23633400]

Takeuchi JK, Mileikovskaia M, Koshiba-Takeuchi K, Heidt AB, Mori AD, Arruda EP, Gertsenstein M, Georges R, Davidson L, Mo R, et al. 2005 Tbx20 dose-dependently regulates transcription factor networks required for mouse heart and motoneuron development. Development. 132:2463–2474. [PubMed: 15843409]

Tam PP, Parameswaran M, Kinder SJ, Weinberger RP. 1997 The allocation of epiblast cells to the embryonic heart and other mesodermal lineages: the role of ingression and tissue movement during gastrulation. Development. 124:1631–1642. [PubMed: 9165112]

Tamura M, Hosoya M, Fujita M, Iida T, Amano T, Maeno A, Kataoka T, Otsuka T, Tanaka S, Tomizawa S, Shiroishi T. 2013 Overdosage of Hand2 causes limb and heart defects in the human chromosomal disorder partial trisomy distal 4q. Hum Mol Genet. 22:2471–2481. [PubMed: 23449628]

Thattaliyath BD, Firulli BA, Firulli AB. 2002 The basic-helix-loop-helix transcription factor HAND2 directly regulates transcription of the atrial naturetic peptide gene. J Mol Cell Cardiol. 34:1335–1344. [PubMed: 12392994]

Thattaliyath BD, Livi CB, Steinhelper ME, Toney GM, Firulli AB. 2002 HAND1 and HAND2 are expressed in the adult-rodent heart and are modulated during cardiac hypertrophy. Biochem Biophys Res Commun. 297:870–875. [PubMed: 12359233]

Tian X, Pu WT, Zhou B. 2015 Cellular origin and developmental program of coronary angiogenesis. Circ Res. 116:515–530. [PubMed: 25634974]

Togi K, Kawamoto T, Yamauchi R, Yoshida Y, Kita T, Tanaka M. 2004 Role of Hand1/eHAND in the dorso-ventral patterning and interventricular septum formation in the embryonic heart. Mol Cell Biol. 24:4627–4635. [PubMed: 15143159]

Togi K, Yoshida Y, Matsumae H, Nakashima Y, Kita T, Tanaka M. 2006 Essential role of Hand2 in interventricular septum formation and trabeculation during cardiac development. Biochem Biophys Res Commun. 343:144–151. [PubMed: 16530167]

Tsuchihashi T, Maeda J, Shin CH, Ivey KN, Black BL, Olson EN, Yamagishi H, Srivastava D. 2011 Hand2 function in second heart field progenitors is essential for cardiogenesis. Dev Biol. 351:62–69. [PubMed: 21185281]

van den Berg G, Abu-Issa R, de Boer BA, Hutson MR, de Boer PAJ, Soufan AT, Ruijter JM, Kirby ML, van den Hoff MJB, Moorman AFM. 2009 A caudal proliferating growth center contributes to both poles of the forming heart tube. Circ Res. 104:179–188. [PubMed: 19059840]

Vandenberg LN, Levin M. 2013 A unified model for left-right asymmetry? Comparison and synthesis of molecular models of embryonic laterality. Dev Biol. 379:1–15. [PubMed: 23583583]

VanDusen NJ, Casanovas J, Vincentz JW, Firulli BA, Osterwalder M, Lopez-Rios J, Zeller R, Zhou B, Grego-Bessa J, La Pompa De JL, et al. 2014 Hand2 is an essential regulator for two Notch-dependent functions within the embryonic endocardium. Cell Rep. 9:2071–2083. [PubMed: 25497097]

VanDusen NJ, Vincentz JW, Firulli BA, Howard MJ, Rubart M, Firulli AB. 2014 Loss of Hand2 in a population of Periostin lineage cells results in pronounced bradycardia and neonatal death. Dev Biol. 388:149–158. [PubMed: 24565998]

Vincentz JW, Barnes RM, Rodgers R, Firulli BA, Conway SJ, Firulli AB. 2008 An absence of Twist1 results in aberrant cardiac neural crest morphogenesis. Dev Biol. 320:131–139. [PubMed: 18539270]

Vincentz JW, Casasnovas JJ, Barnes RM, Que J, Clouthier DE, Wang J, Firulli AB. 2016 Exclusion of Dlx5/6 expression from the distal-most mandibular arches enables BMP-mediated specification of the distal cap. Proc Natl Acad Sci USA. 113:7563–7568. [PubMed: 27335460]

Vincentz JW, Toolan KP, Zhang W, Firulli AB. 2017 Hand factor ablation causes defective left ventricular chamber development and compromised adult cardiac function. PLoS Genet. 13:e1006922. [PubMed: 28732025]

Wang J, Lu Y, Chen H, Yin M, Yu T, Fu Q. 2011 Investigation of somatic NKX2–5, GATA4 and HAND1 mutations in patients with tetralogy of Fallot. Pathology. 43:322–326. [PubMed: 21519287]

Yamagishi H, Yamagishi C, Nakagawa O, Harvey RP, Olson EN, Srivastava D. 2001 The Combinatorial Activities of Nkx2.5 and dHAND Are Essential for Cardiac Ventricle Formation. Dev Biol. 239:190–203. [PubMed: 11784028]

Yutzey KE, Kirby ML. 2002 Wherefore heart thou? Embryonic origins of cardiogenic mesoderm. Dev Dyn. 223:307–320. [PubMed: 11891982]

Zeisberg EM, Ma Q, Juraszek AL, Moses K, Schwartz RJ, Izumo S, Pu WT. 2005 Morphogenesis of the right ventricle requires myocardial expression of Gata4. J Clin Invest. 115:1522–1531. [PubMed: 15902305]

Zhao Y, Samal E, Srivastava D. 2005 Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Nature. 436:214–220. [PubMed: 15951802]

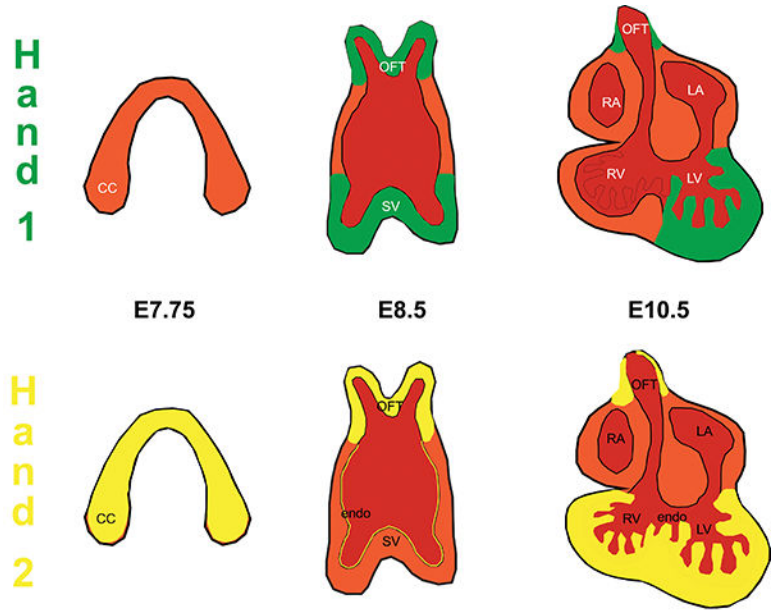

FIGURE 1:

Schematic representation of stages of mammalian heart development with expression of *Hand1* (in green) and *Hand2* (in yellow) as marked at the respective stages. CC: Cardiac Crescent; OFT: Outflow tract; SV: Sinus Venosus; endo: Endocardium; RA: Right Atrium, RV: Right Ventricle; LA: Left Atrium; LV: Left Ventricle.

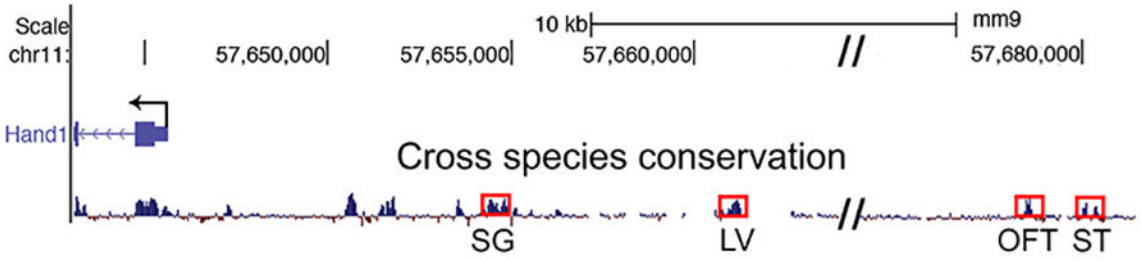

FIGURE 2:

The mouse *Hand1* locus as visualized in http://genome.ucsc.edu (Kent et al. 2002). The 5' region has mammalian conserved regions (red boxes) that have been interrogated for their spatial and temporal specific activity. SG: sympathetic ganglia; LV: left ventricle; OFT: outflow tract; ST: septum transversum.